MICRO AND SEMIMICRO DETERMINATION OF NITROGEN IN HETEROCYCLIC NITROGEN RING COMPOUNDS BY A KJELDAHL METHOD*

By C. L. Ogg, Ruth W. Brand, and C. O. Willits
(Eastern Regional Research Laboratory†, Philadelphia 18, Pa.)

Modifications in the Kjeldahl procedure to increase the range of its application have been numerous, and as Shirley and Becker (8) pointed out, conflicting claims have been made as to the correct procedure for the analysis of compounds containing refractory ring-type nitrogen. Clark (5) claimed that the semimicro adaptation of the Gunning-Arnold-Dyer modification was applicable to practically all animal and vegetable materials, purines, pyrimidines, and pyridine and quinoline derivatives.

The Clark modification, which is the tentative micro Kjeldahl method of the Association of Official Agricultural Chemists (2), uses mercuric oxide as catalyst with 1.5 ml concentrated sulfuric acid and 0.5 g potassium sulfate and a total digestion time of 80–85 minutes. However, Clark stated that some alkaloids and related compounds require a longer digestion time, and that atropine and quinine-type compounds require 2 hours' digestion. Acree (1), in doing collaborative work with this method, was able to obtain only one-fourth of the nitrogen in nicotinic acid with a 30-minute digestion and found that a 2-hour digestion was required for strychnine and quinine hydrobromides and atropine sulfate.

Belcher and Godbert (3) reported satisfactory nitrogen values for nicotinic acid, quinolinic acid, atropine sulfate, picolinic acid, etc., by a semimicro method employing mercuric sulfate and metallic selenium as catalysts and a total digestion time of 45 minutes. . . . However, they state that loss of nitrogen occurs when the digestion time exceeds 75 minutes. This loss probably is caused by the high concentration of selenium used in the digestion mixture since the concentration exceeds the limit found by Bradstreet (4). To avoid this loss an empirical digestion time which may vary with different compounds must be used. Miller and Houghton (6), using a micro method with mercuric oxide as catalyst, found that *l*-tryptophane (sample in solution) required a 6-hour digestion, and consequently they adopted this digestion time as standard for proteins and amino acids.

White and Secor (9) reported 98.5 to 100 per cent recovery of the nitrogen from *l*-tryptophane with Clark's method. Although their nitrogen values for *l*-tryptophane with an 85-minute digestion were close to theory, better values were obtained when the time was increased to 115 minutes.

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† One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

Previous to the studies reported in this paper, all compounds containing ring nitrogen were analyzed in this laboratory by the Dumas method. Since so much time is required to condition the Dumas apparatus and establish the blank when it is not in continuous use, and since a number of samples can be run simultaneously by the Kjeldahl method with a considerable saving in time, a reliable micro Kjeldahl procedure for the analysis of such materials is highly desirable. Consequently, a study of the Kjeldahl method was made in which various catalysts and combinations of catalysts were used and the time of digestion ranged from 1 to 6 hours. The semimicro procedure adopted is substantially the same as the macro procedure of Shirley and Becker, and the results obtained in the digestion-

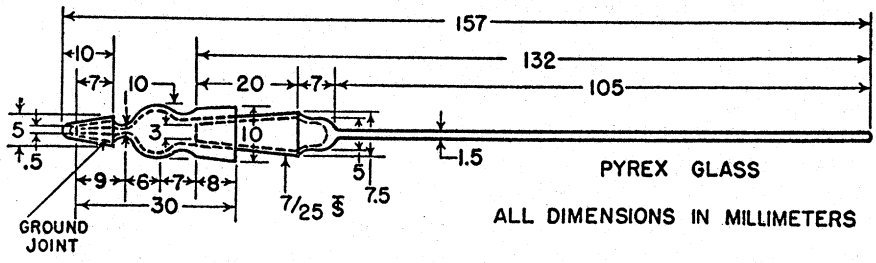

FIG. 1.—Semimicro Charging Tube.

time studies on nicotinic acid on the semi-micro and macro scale are relatively parallel. . . . Since the semimicro procedure and apparatus have been successfully applied to micro analyses, both methods are described.

APPARATUS

(1) *Digestion stand.*—6-unit, gas-heated, with glass manifold to allow for the removal of acid fumes by suction from a water aspirator.

(2) *Kjeldahl flasks.*—30 ml Pyrex glass.

(3) *Distillation apparatus.*—Micro Kjeldahl apparatus, Scientific Glass Apparatus Co. Number M-3704.*

(4) *Steam generator.*—A two-liter, three-necked flask with a 24/40 outer joint on the center neck. The flask contains a heating element consisting of a coiled wire of 25-ohms resistance. The ends of the resistance wire are brazed to 14-gage copper leads which pass out through rubber stoppers in the two outer necks and are soldered to the wires of an insulated extension cord. The rate of steam evolution caused by passing a current through the resistance wire is controlled by a variable resistor of 5 ampere rating. With no heat loss between heating element and the water, the heating can be instantaneously controlled.

(5) *Burettes.*—10-ml graduated in 0.05 ml (semimicro) or 5 ml graduated in 0.01 ml (micro).

REAGENTS

(1) *Catalyst.*—Thoroly mix 150 g powdered potassium sulfate, 10 g of mercuric oxide, and 3 g of powdered metallic selenium.

(2) *Sodium hydroxide-sodium thiosulfate soln.*—Mix 100 ml of 50% sodium hydroxide and 25 ml of 8% sodium thiosulfate soln.

* The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

(3) *Indicator.*—Mix 100 ml of 0.2% methyl red dissolved in 95% ethanol with 50 ml of 0.2% methylene blue also dissolved in 95% ethanol.

(4) *Boric acid.*—Dissolve 4 g boric acid in 100 ml distilled water.

(5) *Standard acids.*—0.02 *N* hydrochloric acid (semimicro) of 0.01 *N* (micro).

PROCEDURE

Weigh a 15–30 mg sample (4–8 mg for the micro procedure) in a long-handled charging tube and transfer to a 30 ml Pyrex Kjeldahl flask. Add 0.65 –0.70 g of the catalyst 3–4 boiling chips, and 2 ml of concd sulfuric acid, rotating the flask as the acid is added so that any sample adhering to the lower portion of the neck will be rinsed into the bulb. Place the flask on the digesting apparatus, heat slowly until carbonization occurs or until all danger of frothing is past, and then adjust the heat so that the sulfuric acid distills ca two-thirds the way up the neck of the flask. Continue the digestion for 4 hours for materials containing ring nitrogen, or 1 hour for more easily digested material such as amines or amides. After the digestion is complete, cool the flask, add 3 ml of water, and again cool the flask to room temp. Condition the distillation apparatus by steaming it out for 2–3 min., and then rinse it with distilled water. Place 10 ml of a 4% boric acid absorbing soln and 3 drops of indicator in a freshly rinsed 125 ml Erlenmeyer flask and place this flask under the tube of the condenser so that the delivery tip is immersed in the soln. Transfer the digestion mixture to the distillation unit with both stopcocks open to prevent any sample from being drawn into the outer chamber. To insure quantitative transfer, rinse the flask with four 2 ml portions of distilled water, or until the rinsings remain yellow on addition of a drop of methyl orange indicator. Add 6 ml of the sodium hydroxide-sodium thiosulfate soln (1 ml in excess of that needed to neutralize the acid), close both stopcocks, and heat the water in the steam generator slowly until the displaced air has mixed the acid and alkali. If the generator is heated too rapidly, the violent reaction which will result from the sudden mixing of the acid and alkali may cause the sample and absorbing soln to be sucked back thru the apparatus. After the acid and alkali are thoroly mixed, turn the current on full to generate the steam at a rapid rate until it reaches the condenser, then reduce the rate so that the steam does not pass the upper half of the condenser. (This should allow 3–4 ml of distillate to be collected per min.) After it has distilled for 3 min., lower the receiving flask so that the delivery tip is above the soln and continue the distillation for 1 min. to rinse the inside of the condenser tube. Rinse the outside of the delivery tip with distd water, dilute the soln to 50 ±5 ml, and titrate with the appropriate standard hydrochloric acid until the soln changes from green to gray or gray-purple. Run a blank determination to correct for nitrogen in the reagents. Use this blank value to correct the volume of acid used in titrating the sample and calculate the percentage of nitrogen.

(Ten milliliters of the 4% boric acid soln, after dilution to 50 ml, is slightly acid to the indicator, but in practice the ammonia from the reagents "blank" has always been sufficient to neutralize the boric acid.)

Hygroscopic solids are dried and weighed in long-handled charging tubes (7) (Figure 1). The tube and cap are made from a 7/25 inner and outer joint with 10 mm of the outer joint (large cap) cut off to reduce the weight. Sealed to the top of the large cap is a smaller inner joint with a capillary opening, which in turn is covered by a ground-glass cap. The sample is placed in the charging tube and dried under vacuum, with only the large cap attached. When the sample is dry, the vacuum is released by admitting dry air to the drying chamber. The charging tube is then

TABLE 1.—*Effect of catalyst and digestion time in the Kjeldahl analyses of nicotinic acid*
Nicotinic Acid, Theory = 11.38% *N*

CATALYST	PER CENT NITROGEN FOUND AFTER DIGESTION TIME OF:											
	1 HOUR			2 HOURS			3 HOURS			4 HOURS		
	Max.	*Min*	*Ave.*	*Max.*	*Min.*	*Ave.*	*Max.*	*Min.*	*Ave.*	*Max.*	*Min.*	*Ave.*
Hg (1 drop)				8.69	7.86	8.33						
HgO (40 mg)				9.30	8.40	8.85	9.91	8.66	9.54	11.26	10.78	11.00
HgO + Se[1]	7.40	5.68	6.54	10.16	9.36	9.74	11.39	10.33	11.08	11.39	11.28	11.36
$CuSO_4$[2] + Se				8.42	7.28	7.95						
HgO + $SeOCl_2$ (1 drop)	4.98	4.88	4.93	8.82	7.22	8.26	11.02	8.10	9.66	11.35	11.28	11.31
HgO + $SeOCl_2$ (2 drops)				11.12	8.01	10.04	10.76	10.39	10.58			
$CuSO_4$ + $SeOCl_2$ (1 drop)				7.54	6.11	6.58						
HgO + Se + $CuSO_4$	5.55	4.55	5.05	10.34	7.78	8.99				11.28	11.07	11.16
HgO + $CuSeO_3$ (5 mg)				8.42	7.28	7.95						
Clark's Method (5)	5.31	3.58	4.45									

[1] Selenium-coated granule.
[2] 0.15 g. $CuSO_4 \cdot 5H_2O$.

removed, and the capillary is immediately closed with the small cap and allowed to come to room temperature before weighing.

Moderately volatile or hydroscopic liquid samples are weighed in capsules made by sealing the tube of a 5/12 inner joint just below the joint. For weighing and introducing into the digestion flask the capsule is inserted loosely in a holder consisting of a 5/12 outer joint whose tube serves as a handle. After the capsule and holder are tared, the sample is introduced in the capsule with either a hypodermic syringe or a medicine dropper having a capillary tip, and the capsule is placed in the holder. The capsule with the sample is weighed and dropped into a digestion flask containing the acid and catalyst by gently tapping the holder against the neck of the flask. After digestion, the mixture is transferred to the distilling apparatus and the flask is rinsed as usual. The capsule, which is caught in the funnel, is removed with tweezers, held in an inverted position over the funnel, and rinsed with 2 ml of water from a hypodermic syringe. The needle is bent in a V-shape so that the rinse solution which drains down is transferred to the funnel. Very viscous liquids, gums and resins are introduced into the flask in porcelain boats. Hygroscopic samples are dried and weighed in weighing pigs.

RESULTS

Nicotinic acid was chosen for use in studies of catalyst and digestion time, since it contains a ring-type nitrogen which has proved difficult to obtain by the Kjeldahl method, is easily obtained in pure form, and is a non-hygroscopic solid. Several catalysts and combinations of catalysts were used, and the digestion time was varied from 1 to 4 hours. . . . The amount of catalyst in each combination was the same unless otherwise noted. Table 1 shows the results obtained with the various digesting conditions.

A 2-hour digestion period was used in comparing the relative effectiveness of the various catalysts and combinations, since it was not sufficiently long to give complete recovery with any of the catalysts. The results obtained under these conditions indicate the most effective catalyst to be a combination of mercuric oxide and selenium. Mercuric oxide and selenium oxychloride (2 drops) gave a higher nitrogen value in 2 hours than did the mercuric oxide and selenium, but as Bradstreet (4) has shown, the ratio of selenium to sulfuric acid was so high that loss of nitrogen was almost certain, and as expected, the values obtained after the 3-hour digestion were lower than those obtained with mercury and selenium. Increasing the amount of selenium increased the rate of conversion of nitrogen to ammonia, but apparently nitrogen was lost when the selenium content was raised much above 10 mg per ml of sulfuric acid. The results obtained for the 3-hour digestion with mercuric oxide and selenium show that in some cases theoretical values were obtained but the recoveries were not

TABLE 2.—*Kjeldahl nitrogen analyses of several nitrogenous compounds*

COMPOUND	CATALYST	DIGESTION TIME	NITROGEN			
			FOUND		AVE.	THEORETICAL
		hours	*per cent*	*per cent*	*per cent*	*per cent*
N (α-pyridyl) nicotinamide	HgO+Se	3	20.80			
		3	20.85	20.67	20.77	21.10
		4	21.02	21.03		
		4	21.02	21.07	21.04	21.10
Nicotine	HgO+Se	1	13.74	14.79	14.27	17.27
		2	16.93	16.46	16.70	17.27
		3	17.22	17.23		
		3	17.31	17.19	17.24	17.27
		6	17.20	17.22	17.21	17.27
8-Hydroquinoline	HgO+Se	4	9.64	9.69	9.67	9.65
Tryptophane	HgO+$SeOCl_2$	4	13.74	13.72		
		4	13.75	13.77	13.75	13.72
Tryptophane	HgO+Se	1	13.17	13.05	13.11	13.72
		2	13.75	13.70	13.73	13.72
		4	13.65	13.66		
		4	13.64	13.69	13.67	13.72
Acetonicotyrine	HgO+$SeOCl_2$	4	11.34	12.88	12.11	13.73
		6	13.66	13.51	13.59	13.73
Nicotine picrate[1]	HgO+Se	4	18.11	18.05		
		4	17.89	18.16	18.05	18.06
Nicotine picrate[2]	HgO+Se	4	18.01	17.98	18.00	18.06
S-benzyl thiuronium chloride	HgO+Se	1	13.78	13.83		
		1	13.79	13.79	13.80	13.82
Acetanilide	HgO+Se	1	10.35	10.31	10.33	10.36

[1] Sample treated with salicylic acid and sodium thiosulfate. 3 ml. H_2SO_4 used.
[2] Sample digested for 2 hours with carbon prior to regular 4-hour digestion. 3 ml. H_2SO_4 used.

consistent, whereas with 4 hours' digestion concordant results were obtained which were close to the theoretical values. With the catalyst mixture recommended complete recovery of the nitrogen was obtained in 4 hours and no loss of nitrogen was detected with a 6-hour digestion. Consequently, a 4-hour digestion period was tentatively adopted as standard for all materials containing ring-type nitrogen. This digestion time is 1 hour longer than was indicated by Shirley and Becker's work (8), in which macro procedures were used. The difference may be due to the type of

heater used during the digestion, that is, electrical, versus gas heaters. Nitrogen was determined in several materials in addition to nicotinic acid by the method described to test its applicability to several types of compounds having ring nitrogen. The results are reported in Table 2.

All the compounds studied yielded their nitrogen after a 4-hour digestion with mercuric oxide and selenium except acetonicotyrine, which required a 6-hour digestion for the values to approach theory.

Nicotine, whose structure differs only slightly from that of nicotyrine, needed only a 3-hour digestion, thus indicating the difficulty that may be experienced in attempting to predict the digestion time required from the structure of the compound to be analyzed. Doubling the digestion time required for nicotine did not significantly lower the nitrogen values, proving that if loss of nitrogen does result from the use of selenium catalyst it is negligible. As would be expected, a 1-hour digestion was sufficient for acetanilide and S-benzyl thiuronium chloride, since they do not contain ring nitrogen. Tryptophane yielded its nitrogen after a 2-hour digestion. This is in agreement with the observations of White and Secor (9).

The results obtained show that in most cases a 4-hour digestion with mercuric oxide and selenium as catalysts is sufficiently long to obtain the nitrogen from heterocyclic nitrogen ring compounds. Apparently a few compounds require at least a 6-hour digestion. It is easy to detect these materials, however, since the incomplete 4-hour digestion usually is accompanied by discordant results.

SUMMARY

A semimicro Kjeldahl method is presented for the determination of nitrogen in compounds containing heterocyclic ring nitrogen. This method has proved successful in the analysis of micro-sized samples. With a mixture of mercury and selenium, the best catalyst found, a minimum digestion time of 4 hours is required. For compounds not containing ring nitrogen, the digestion time can be shortened to 1 hour.

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